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# Expression of *bcl*-2 in Fetal Tissues Suggests a Role in Morphogenesis

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***We have studied the distribution of the* bcl-*2 protein in fetal tissues, in an effort to uncover patterns of expression that may elucidate the potential role of* bcl-*2 during development. We find that* bcl-*2 is expressed in many hematolymphoid and non-hematolymphoid tissues, most abundantly in placental trophoblast. In tissues of endocrine and neural derivation and in stem-cell populations of colonic and some stratified epithelia,* bcl-*2 seems to be involved in tissue homeostasis. However, in developing proximal nephrons of the kidney and other sites characterized by inductive interactions between epithelium and mesenchyme,* bcl-*2 is apparently involved in morphogenesis, possibly by mediating the formation of condensations of cells that are "committed" to the formation of more differentiated structures. The distribution of* bcl-*2-protein expression in fetal tissues is consistent with its previously described role in promoting cell survival, presumably by preventing apoptosis in lymphoid and other tissues where cell death represents an active regulatory process. Expression of* bcl-*2 protein is more widespread in fetal than adult tissues. Our observations therefore represent supportive evidence for the importance of inducible cell survival as a regulatory process in normal homeostasis and morphogenesis in many fetal tissues and structures. (Am J Pathol 1993, 142:743–753)***

The *bcl*-2 proto-oncogene on chromosome 18 has been implicated in the pathogenesis of a large proportion of non-Hodgkin's lymphomas, particularly those with a follicular architecture.[1–7] In these lymphomas, *bcl*-2 expression is deregulated by the t(14;18)(q32;q21) chromosomal translocation,[8] which juxtaposes the *bcl*-2 gene with long-range transcription control elements associated with the immunoglobulin heavy chain locus. Although recent *in vitro* evidence suggests that *bcl*-2 may normally serve a regulatory function in permitting specific cell types to avoid undergoing apoptosis in tissues where preprogrammed cell death may represent an active control mechanism,[9] the role of *bcl*-2 in normal physiology remains unclear.

In order to gain insight into the physiological function of *bcl*-2, we studied the distribution of its expression in the tissues of fetuses at approximately 12, 15, and 22 weeks of gestation. The availability of a highly specific monoclonal antibody against the *bcl*-2 gene product permitted us to carry out immunohistochemistry on sections from paraffin-embedded and snap-frozen tissue specimens, as well as semi-quantitative studies using sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Our results confirm that *bcl*-2 is expressed in a wide variety of fetal tissues, including those of hematolymphoid, epithelial, neural, endocrine, and mesenchymal type. Some of these tissues also have been recently reported to show expression in adults, supporting a role for *bcl*-2 in tissue homeostasis. However, we also detected the gene product in condensations of cells committed to the formation of more complex structures, such as hair follicles in the skin and glomeruli in the kidney, suggesting that *bcl*-2 may be involved in certain aspects of morphogenesis. In consideration of the role of *bcl*-2 in maintenance of B cell survival, our observations are consistent with the possibility that focal modulation of the rate of cell death may be more prevalent among fetal tissues as a morphogenetic mechanism than previously believed.

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## Materials and Methods

### Tissue Procurement

Samples representing a wide variety of tissues were collected from 12-, 15-, and 22-week fetuses within 30 minutes of therapeutic abortion. Sections of tissue roughly 3-mm thick were fixed in 10% buffered formaldehyde for 7 hours then subjected to routine processing and paraffin embedding. Samples of several tissues from the 22-week fetus, were snap-frozen in a bath of ethanol and dry ice. Unstained sections from each sample were cut and mounted on glass slides, and one section from each was stained with hematoxylin and eosin for morphological evaluation.

### *Immunological Detection of* bcl-2 *Protein*

Tissue culture supernatant containing the mouse-derived monoclonal antibody to *bcl-2* used in these studies (clone 100) was a generous gift of Dr. David Mason, Oxford University, U.K.. Its production and characterization have been described in detail elsewhere.[10] This reagent was diluted 1:4 in phosphate-buffered saline as a primary antibody for paraffin-section immunohistochemistry and 1:40 for frozen section studies. The antibody at these dilutions gives the expected staining pattern in adult tonsil or lymph node. The details of the avidin-biotin detection system used in our laboratory for immunohistochemistry have been published previously.[11] Briefly, a 40-minute incubation with the primary antibody was followed by sequential incubations with a biotinylated, goat-derived antibody against mouse immunoglobulin, streptavidin-conjugated horseradish peroxidase, and finally diaminobenzidine as a chromogen, with phosphate-buffered saline washes between each step. Because of the possibility of antigen loss or alteration by formaldehyde fixation and tissue processing, the findings on paraffin-section immunohistochemistry were compared with those from snap-frozen tissue for tissues from which paraffin-embedded and frozen specimens were prepared (cerebral cortex, thymus, lung, myocardium, adrenal, spleen, kidney, skeletal muscle, skin, liver, placenta, extrafetal membranes, and umbilical cord). Immunostaining to detect keratin (antibody AE1, Boehringer Mannheim, Indianapolis, IN), chromogranin (anti-chromogranin A, Dako, Carpinteria, CA), and the proliferative nuclear antigen ki-67 (antibody ki-67, Dako) was performed on selected sections. Negative controls were performed on a section from each sample by omitting the primary antibody and by substituting irrelevant, isotype-matched, mouse-derived monoclonal antibodies as primary antibodies (PD-7 for non-hematolymphoid tissues and *Ber*H2 for lymphoid tissues, both obtained from Dako).

SDS polyacrylamide gel electrophoresis was carried out using standard methods.[12] Briefly, snap-frozen tissue specimens were thawed at room temperature, and 5 to 25 mg of each was excised, cut into minute fragments using a razor blade, and weighed on an analytical balance. Tissue fragments were then suspended in 10 volumes of a buffer containing 2% SDS, 100 mmol/L dithiothreitol, and 60 mmol/L Tris, pH 6.8. The resulting suspension was boiled for 10 minutes and passed several times through a 25-gauge hypodermic needle to shear genomic DNA. The total protein content of each lysate was determined colorometrically using reagents obtained commercially (Bio-Rad Laboratories, Richmond, CA), and an aliquot containing 150 µg of protein was combined with 1/5 volume of concentrated loading buffer (100 mmol/L Tris, pH 6.8, 200 mmol/L dithiothreitol, 4% SDS, 0.2% bromphenol blue, and 20% glycerol), heated to 80 C for 10 minutes and loaded onto a discontinuous 12% polyacrylamide gel containing 0.1% SDS.[13] After electrophoresis, proteins were transferred electrophoretically onto a nitrocellulose membrane. Non-fat dried milk was used to block nonspecific antibody binding to the membrane, and free avidin followed by free biotin were used to block endogenous avidin–binding activity. *bcl-2* protein was detected using anti-*bcl-2* at a dilution of 1:40 and a detection system similar to that used for immunohistochemistry. Even protein loading was confirmed by Coomasie staining of a duplicate gel.

## Results

Immunostaining of tissue sections revealed the presence of *bcl-2* protein in many tissue types in variable abundance (Table 1). *bcl-2* was expressed in cells in the basal layers of several stratified or pseudostratified epithelia (esophagus, palate, trachea, vagina, conjunctiva) and, weakly, at the bases of colonic crypts (Figure 1A). In the lung, staining was limited to a population of flattened, elongated cells that, in interrupted patches, formed a layer one-cell thick immediately surrounding the epithelium of the developing tracheobronchial tree (Figure 1B). The non-epithelial nature of these cells was supported by their lack of reactivity with an anti-keratin monoclonal antibody that stained the epithelial cells intensely (data not shown). *bcl-2* staining patterns in the epithelial cells of the exocrine pancreas and parotid gland were similar, with staining limited to some cells in larger ducts. In the pancreas, the small number of

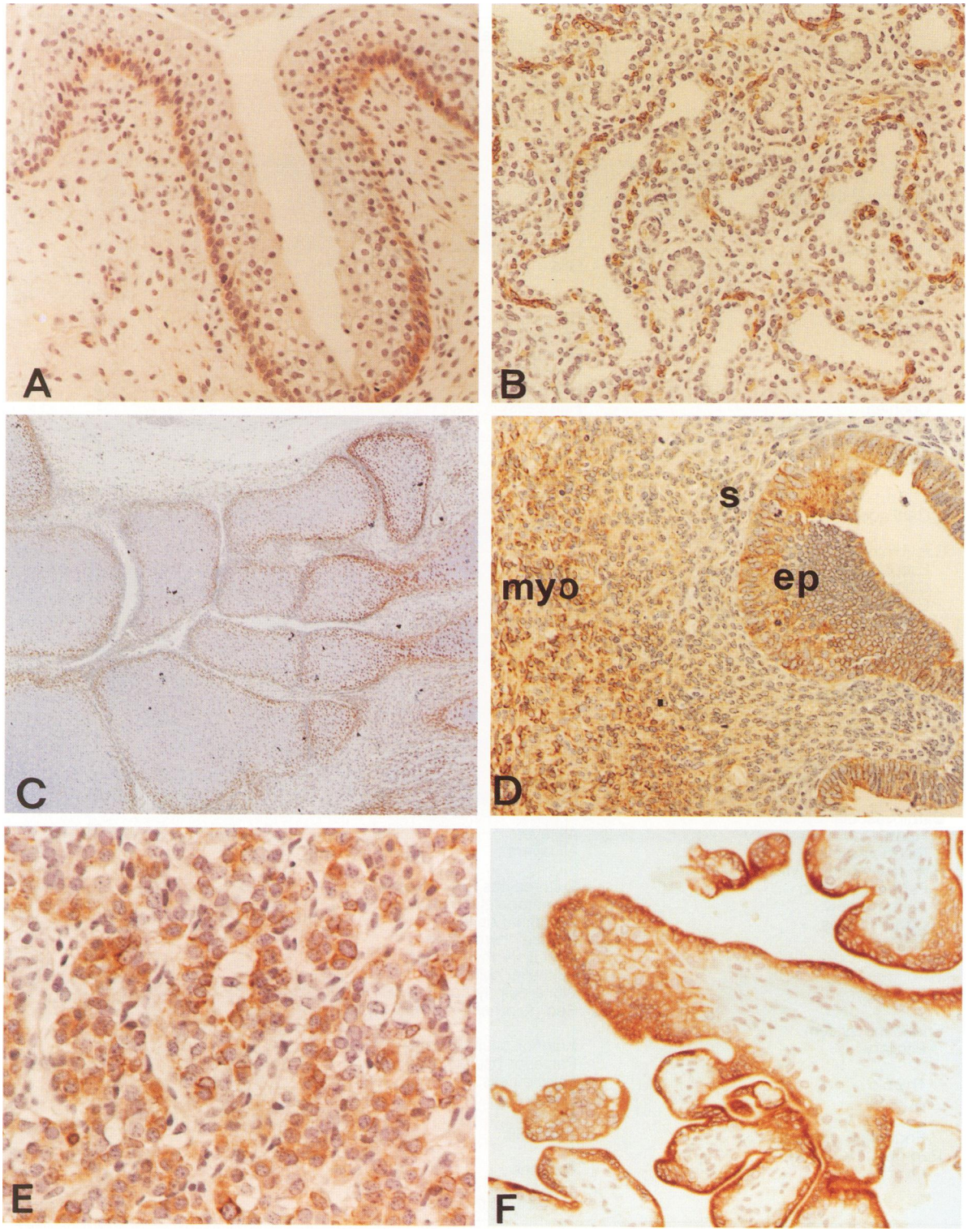


**Figure 1.** *Patterns of* bcl-*2 staining.* **A:** *The stratified squamous epithelium of the palate illustrates restriction of* bcl-*2 expression to the basal cell layer. A similar staining pattern was seen in several other complex epithelia (anti-*bcl-*2,* ×*200).* **B:** bcl-*2 expression in the fetal lung was limited to flattened, elongated mesenchymal cells immediately adjacent to the epithelium of developing air spaces (anti-*bcl-*2,* ×*200).* **C:** *In the cartilage of incipient tarsal and metatarsal bones, shown here, as in cartilagenous masses in several other sites,* bcl-*2 expression was limited to the perichondrial region (anti-*bcl-*2,* ×*100).* **D:** *In the fetal uterus, strong* bcl-*2 expression was present in endometrial epithelium (ep) and myometrium (myo). Expression in endometrial stroma (s) was weaker (anti-*bcl-*2,* ×*200).* **E:** bcl-*2 staining of variable intensity was present in cells of the adenohypophysis (anti-*bcl-*2,* ×*400).* **F:** Intense bcl-*2 staining was noted in the syncytial trophoblast of chorionic villi (anti-*bcl-*2,* ×*200).*

**Table 1.** *Expression of* bcl-*2 Proto-Oncogenic Protein in 22-Week Fetal Tissues*

| Organ/tissue | Cell type | Staining* intensity |
|---|---|---|
| Nervous system | | |
| Cerebral cortex | Neurons | 0–1† |
| | Glia | 0 |
| Spinal cord | Anterior & posterior horn | 2 |
| | Ependyma | 3 |
| | Glia | 0 |
| | Meninges | 0 |
| Peripheral ganglia | Neurons | 0–3† |
| Peripheral nerve | Schwann cells | 2 |
| | Axons/sheaths | 1 |
| Optic nerve | Nerve bundles | 0 |
| | Glia | 1 |
| Eye and ear | | |
| Retina | | 0–3† |
| Conjunctiva | Epithelium | 1‡ |
| Cornea | Epithelium | 0–1§ |
| | Stroma | 2 |
| | Endothelium | 2 |
| Sclera | Fibroblasts | 2 |
| Cochlea | Hair cells | 3 |
| Musculoskeletal system | | |
| Skeletal muscle | Fibers | 0–1†§ |
| Bone | Osteocytes | 2 |
| | Osteoblasts | 0 |
| | Periostium | 0–2‖ |
| Cartilage | Chondrocytes | 0 |
| | Perichondrium | 2 |
| Gastrointestinal system | | |
| Esophagus | Epithelium | 1‡ |
| Stomach | Epithelium | 0–2† |
| Colon | Epithelium | 1§ |
| Smooth muscle | Muscularis mucosae | 2 |
| | Muscularis propria | 1 |
| Liver | Hepatocytes | 0 |
| | Bile ducts | 0–1§ |
| Parotid gland | Epithelium | 0–1† |
| | Mesenchyme | 0 |
| Exocrine pancreas | Acini | 0 |
| | Duct epithelium | 1 |
| Respiratory system | | |
| Tracheobronchial tree | Epithelium | 0 |
| | Mesenchyme | 0–2§ |
| Cardiovascular system | | |
| Heart | All layers | 0‖ |
| Blood vessels | All layers | 0 |
| Hematolymphoid system | | |
| Peripheral blood | Erythrocytes, granulocytes | 0 |
| Bone marrow | Hematopoietic cells | 0¶ |
| Lymph nodes | Lymphoid cells | 0 |
| Thymus | Cortical thymocytes | 0–2† |
| | Medullary thymocytes | 2 |
| | Epithelial cells | 0–2† |
| Spleen | White pulp lymphocytes | 1 |
| | Red pulp hematopoietic cells | 0–1† |
| Endocrine system | | |
| Pituitary | Anterior pituitary cells | 0–2† |
| | Posterior pituitary cells | 0 |
| Thyroid | Follicle cells | 1–2† |
| Adrenal | Cortex | 0 |
| | Medulla | 0–2† |
| Endocrine pancreas | Islet cells | 2 |
| Reproductive system | | |
| Ovary | Oocytes | 0 |
| | Stroma | 0 |
| | Surface epithelium | 1 |

*0 = no staining; 1 = staining weaker than spinal cord neurons; 2 = moderate staining, roughly same intensity as spinal cord neurons; 3 = intense staining stronger than of spinal cord neurons.
†Variability in staining intensity from cell to cell.
‡Epithelial staining in basal layer only.
§Intensity of tissue staining varies according to anatomical location.
‖Periostium in a few locations and endothelium and intima of meningeal vessels show staining of intensity 2.
¶Rare cells show staining of intensity 2.

Table 1. *Continued*

| Organ/tissue | Cell type | Staining* intensity |
|---|---|---|
| Fallopian tube | Epithelium | 0 |
| | Stroma | 2 |
| Uterus | Endometrial epithelium | 2 |
| | Endometrial stroma | 1 |
| | Myometrium | 2 |
| Urinary system | | |
| Kidney | Developing proximal nephrons | 2 |
| | Glomeruli | 0–2§ |
| | Tubular epithelium | 0–2§ |
| Ureter | Epithelium | 0 |
| | Muscularis | 2 |
| Skin | | |
| Skin | Epidermis | 0 |
| | Hair follicle | 1–2§ |
| | Sebaceous glands | 1 |
| | Dermis | 0–2§ |
| Extrafetal tissues | | |
| Placenta | Trophoblast | 3 |
| | Mesenchyme of villi | 0 |
| Umbilical cord | Mesenchyme | 0 |
| Membranes | Chorion | 0 |
| | Amnion | 0 |
| Maternal tissues | | |
| Endometrium | Epithelium | 2 |
| | Decidua | 0 |

mesenchymal cells that were present between theepithelial components also showed immunostaining. Staining was noted in the condensation of mesenchymal cells that constituted the perichondrium of the cartilagenous masses of the auricle, trachea, and some other sites but, except for a small number that were located immediately deep to the perichondrium, chondrocytes themselves were generally unstained (Figure 1C). Periosteum showed staining less frequently, but all osteocytes were stained. The fetal endometrium and myometrium strongly expressed *bcl-2* (Figure 1D). Immunoreactivity was also present in several endocrine tissues (anterior lobe of pituitary, thyroid, pancreatic islets, Figure 1E), and staining in the gastric epithelium was limited to scattered cells in the crypts, with a similar distribution and appearance to gastric neuroendocrine cells, as demonstrated by anti-chromogranin immunostaining (data not shown). The placental syncytial and cytotrophoblast showed intense *bcl-2* staining (Figure 1F).

In the skin of the 12-week fetuses, in which the epidermis is comprised of only 2 cell layers, *bcl-2* was expressed in the basal layer only. In 15- and 22-week fetuses, staining was predominantly in the developing skin adnexa, although faint staining was present in occasional basal keratinocytes. Staining was most intense in the basal cells of epidermal buds in the 15-week fetus and, in more mature hair follicles of the 22-week fetus, in the epithelial cells of the isthmus and the cells of the mesenchymal core (Figure 2, A, B, and C). Immunostaining with ki-67 indicated the proliferative fraction to be small in the mesenchymal core and large in the basal cells of the hair bulb (Figure 2C, inset). Neural tissues frequently expressed *bcl-2* (cortical and spinal neurons, ependymal cells, peripheral ganglion cells including the ganglion cell layer of the retina, Schwann cells, Figure 3, A to D).

In the kidney, *bcl-2* was detected in condensations of mesenchymal cells associated with developing subcapsular nephrons (Figure 4). The glomeruli of more deeply situated, mature nephrons showed less prominent *bcl-2* expression. Comparison of staining for *bcl-2*, ki-67, and keratin on consecutive frozen sections of kidney showed a roughly reciprocal distribution of *bcl-2* protein and ki-67 in some areas and the absence of keratin expression in the strongly *bcl-2*-expressing subcapsular condensations of epithelioid cells (Figure 5, A to C).

Strong staining was noted in the hair cells of the inner ear in the 22-week fetus and in sub-populations of neuroepithelial cells in the developing cochlear duct in the less mature fetuses (Figure 6). *bcl-2* was expressed in most cells of the thymic medulla and in fewer cells of the thymic cortex. However, only scattered immunoreactive cells were present in lymph nodes, which are devoid of lymphoid follicles during normal fetal development. In the spleen, most lymphocytes in the white pulp were stained, as well as scattered, probably hematopoietic cells in the red pulp. Morphological preservation was not adequate to permit recognition of specific cell lineages or maturational stages. Finally, *bcl-2* protein was present in the muscularis mucosae of the gastrointestinal tract and in the cornea and sclera of the eye.

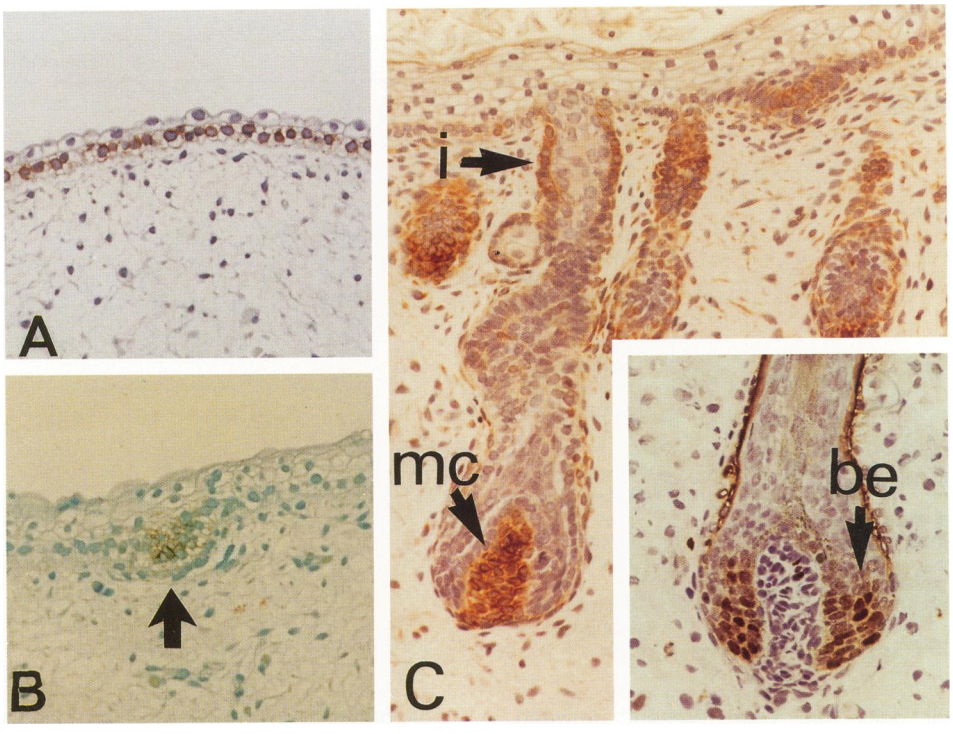


**Figure 2.** bcl-*2 expression in hair follicles.* **A:** *The skin of a 12-week fetus showing restriction of* bcl-*2 expression to the basal layer of the 2 epidermal cell layers (anti-*bcl-*2,* ×*200).* **B:** *In the 15-week fetus, epidermal* bcl-*2 expression was most prominent in the condensed cells constituting the epidermal buds of incipient hair follicles (arrow) (anti-*bcl-*2,* ×*200).* **C:** *In mature hair follicles of the 22-week fetus,* bcl-*2 expression was most prominent in cells of the mesenchymal core (mc) and epithelial cells in the isthmus (i) (anti-*bcl-*2,* ×*200). Note staining of scattered dermal cells and the paucity of staining in the epidermis proper. Frozen-section staining for the proliferative nuclear antigen ki-67 shows a small mitotic fraction in the mesenchymal core, contrasting with abundant mitotic cells among basal epithelial cells (be) of the hair bulb (inset) (ki-67,* ×*200).*

For tissues on which a comparison of paraffin- and frozen-section immunostaining was possible (see Materials and Methods), similar results were obtained with the exception of the cerebral cortex and spleen, where staining was more intense and present in a greater number of cells on frozen tissue sections. The pancreas was examined on the frozen section only.

SDS polyacrylamide gel electrophoresis with Western blot analysis showed the expected single band at roughly 25 kd, characteristic of *bcl-2* oncogenic protein, from tissue lysates of thymus, spleen, brain, kidney, placenta, and lung from the 22-week fetus (Figure 7). The varying intensity of the bands showed *bcl-2* to be most abundant in placenta, followed by kidney, brain, spleen, and thymus. Only a very faint band was present in the lane corresponding to lung, and no *bcl-2* protein was detectable from the liver. Extremely faint bands in the higher molecular weight range were present in every lane and were attributed to incompletely blocked avidin-binding activity because they disappeared when a secondary antibody directly conjugated to horseradish peroxidase was used.

## Discussion

Immunohistochemical studies have shown a compelling correlation between the topographical distribution of *bcl-2*-expressing cells and functional compartments within lymphoid tissues. Thus, in sections of reactive tonsil or lymph node, *bcl-2* staining was most intense in primary lymphoid follicles or the mantle zones of secondary follicles, structures that are comprised largely of long-lived, recirculating lymphocytes.[10,14,15] Recent experimental work has shed light on the role played by *bcl-2* in selection of centrocytes in germinal centers during the antibody response to T cell–dependent antigens.[16] The evidence supports a process whereby centrocytes in the "light zone" of the germinal center are rescued from a commitment to apoptosis if, after somatic mutation in rearranged immunoglobulin variable region genes, the immunoglobulin expressed on the cell surface is capable of high-affinity binding to the antigen presented on the surface of follicular dendritic cells. The binding of surface immunoglobulin apparently triggers *bcl-2* expression in the centrocyte, whereupon it probably moves out of the germi-

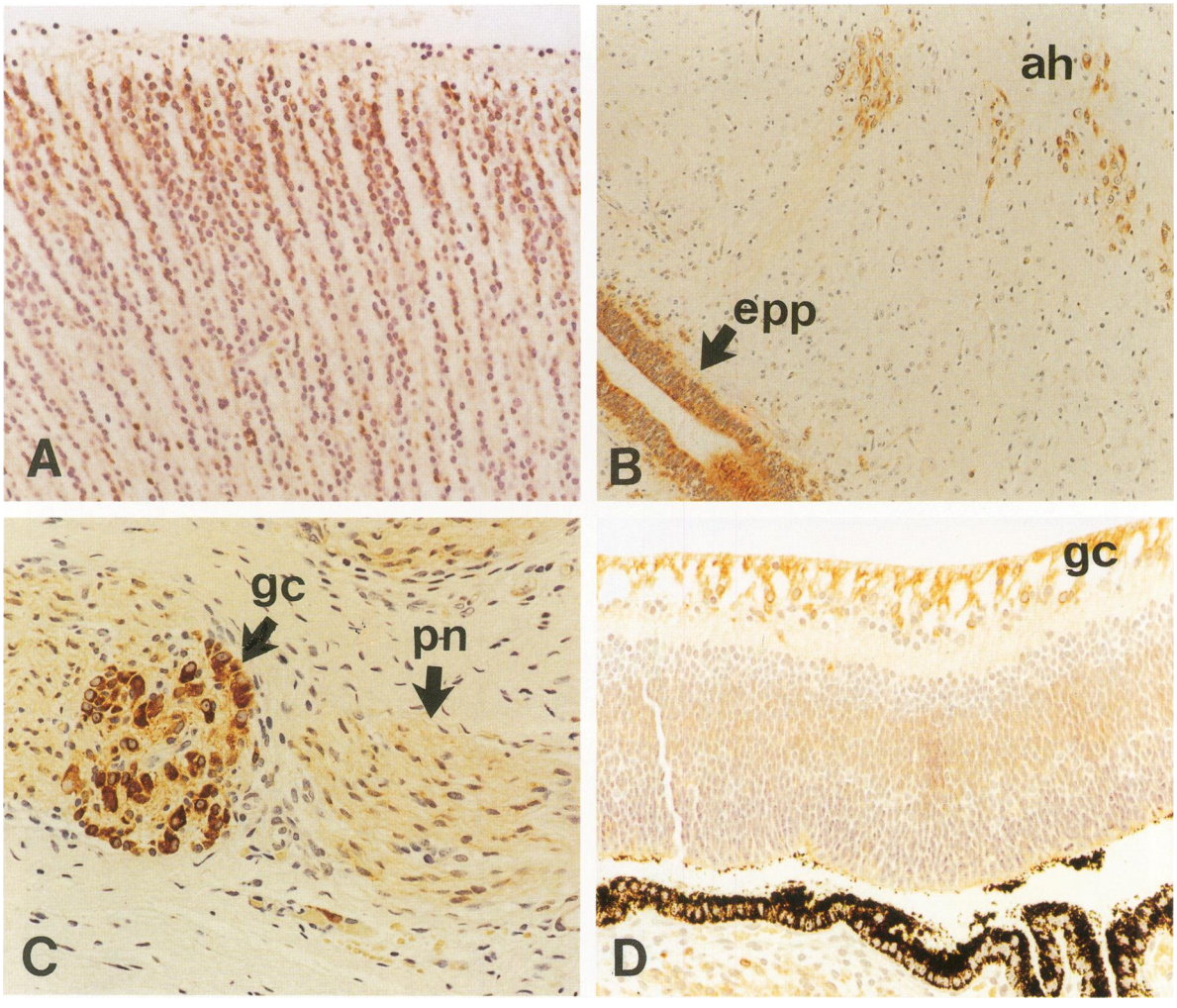


**Figure 3.** bcl-*2 expression in neural tissues.* **A:** *In the cerebral cortex,* bcl-*2 was expressed predominantly by immature neurons near the surface (anti-*bcl-*2,* ×*200).* **B:** *A section from the spinal cord shows strong staining for* bcl-*2 in ependymal cells (epp) and neurons of the anterior horn (ah) (anti-*bcl-*2,* ×*200).* **C:** *Ganglion cells (gc) in a pelvic ganglion show strong staining for* bcl-*2. Also note perinuclear staining of Schwann cells in a peripheral nerve (pn) (anti-*bcl-*2,* ×*200).* **D:** bcl-*2 expression in the retina varied between layers. Note that the most intense staining was in the innermost two layers, namely, the nerve fiber and ganglion cell (gc) layers (anti-*bcl-*2,* ×*200).*

nal center to become a long-lived plasmablast or memory cell. Among lymphocytes of the thymic cortex, most of which are destined to die as a result of negative selection against anti-self clones,[17] only rare cells show *bcl*-2 expression, whereas in the medulla, which is largely comprised of the survivors of the selection process, *bcl*-2 is expressed by the majority of cells.

In addition to growth and diversification, normal fetal development requires differential induction of cell death. Therefore, the current study of the topographical distribution of *bcl*-2 expression in fetal tissues was undertaken with the expectation that consistent patterns of *bcl*-2 gene expression would provide further insight into the physiological role of this unique protein. In a recent study, *bcl*-2 expression was examined immunohistochemically in a variety of adult tissues.[18] The protein was detectable in thymus, hematopoietic cells, endocrine, or hormonally regulated tissues that undergo hyperplasia and involution, and the stem cell population of several complex epithelia. It was concluded that expression of *bcl*-2 protein is topographically restricted in tissues characterized by apoptotic cell death.

As in the adult, we detected *bcl*-2 expression in the fetus in cells in the basal or regenerative regions of several complex epithelia, suggesting that *bcl*-2 may serve to maintain the stem-cell pool by allowing certain cells to avoid following a program of postmitotic differentiation with eventual senescence and death.

The nervous and endocrine systems are closely related embryologically and anatomically. Furthermore, both function to coordinate body processes through chemical signal-response mechanisms. In the endocrine system, trophic effects are generally

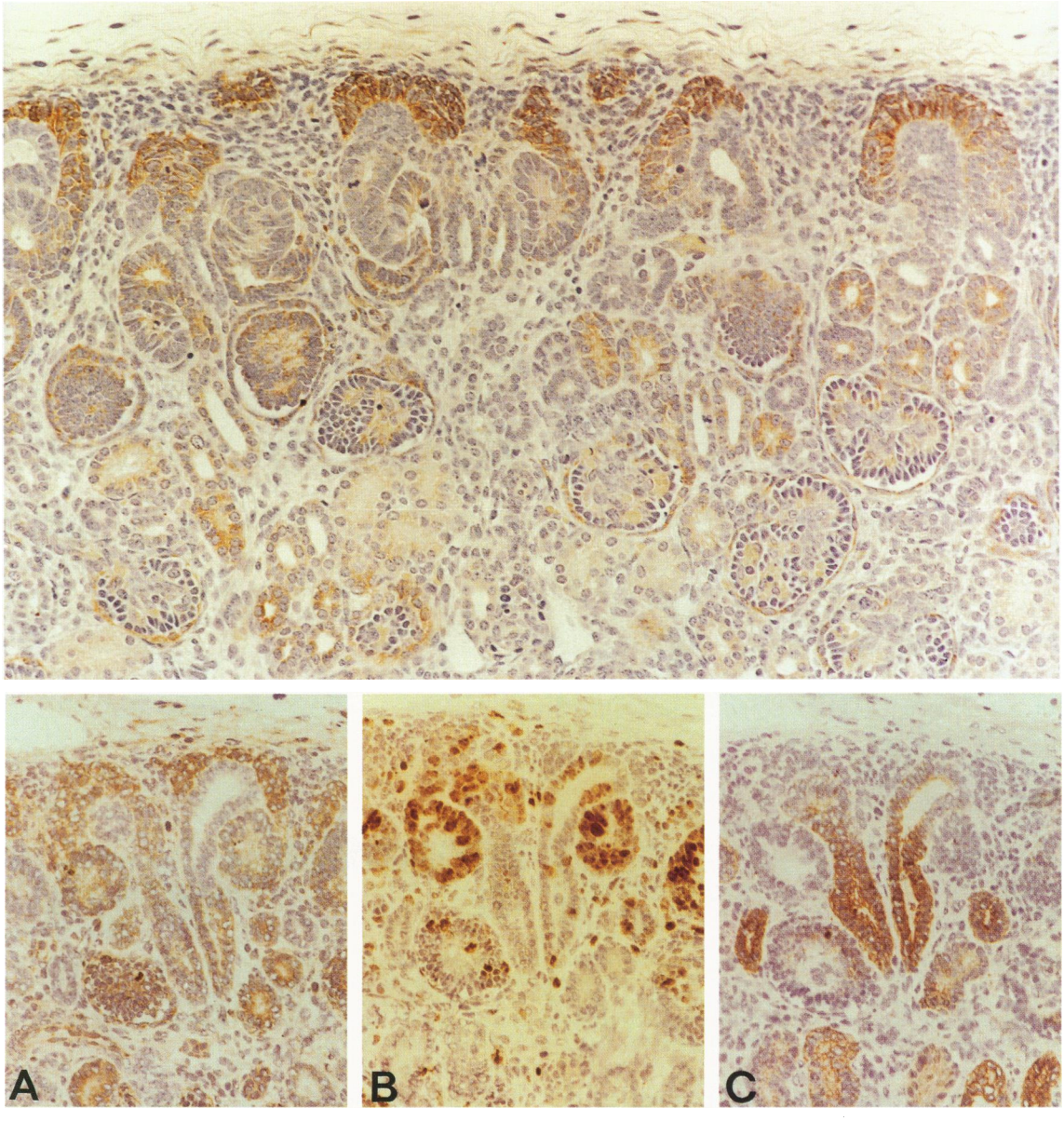


**Figure 4.** bcl-*2 expression in the fetal kidney. Note strong staining for* bcl-*2 in cohesive-appearing cell groups adjacent to tubular structures in the subcapsular region of the 22-week fetal kidney.* bcl-*2 expression seems to persist, although less abundantly, in more deeply situated mature glomeruli and the epithelium of some tubules (anti-*bcl-*2,* ×*200).*

**Figure 5.** *Correlation of bcl-2 expression, mitotic activity, and keratin expression in fetal kidney. Consecutive frozen sections of kidney comparing distribution of bcl-2 protein* **(A)**, *mitotically active cells as detected with the ki-67 monoclonal antibody* **(B)**, *and keratin expression detected with the AE1 monoclonal antibody* **(C)**. *Note the low mitotic fraction and the absence of keratin expression in cohesive-appearing cell groups that stain for* bcl-*2 (*×*200).*

mediated by specific hormones, but metabolites and electrolytes sometimes exert effects directly.[19] The finding that *bcl-2* expression is prevalent in endocrine cells may indicate a role for *bcl-2* in mediating some of the effects of trophic mediators. It is worth considering, in this context, that even with a rate of cell turnover in an organ of only 1%/day, complete abolition of cell death would cause the organ to double in mass every 80 days.[20]

The most abundant expression of *bcl-2* in the tissues we examined is in the placenta, where the protein was predominantly localized to the trophoblast. Staining was as intense in the cytotrophoblast, which predominates at 12 and 15 weeks, as in the syncytial trophoblast, which predominates at 22 weeks. The syncytial trophoblast arises from the cytotrophoblast and has numerous important endocrine functions. Therefore, *bcl-2* theoretically could mediate trophic effects operating as part of a currently uncharacterized endocrine feedback loop. The trophoblast also constitutes the interface between the fetal and maternal circulations; therefore, *bcl-2* protein might play a

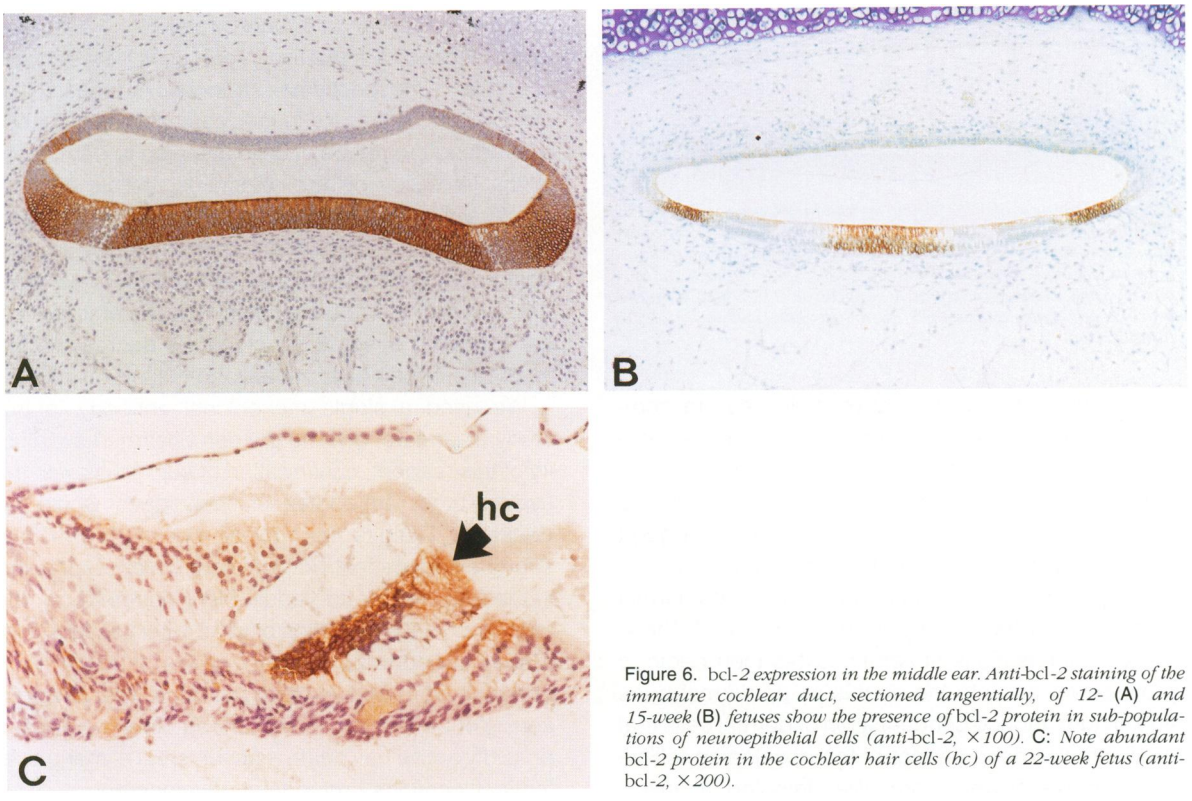


Figure 6. bcl-*2 expression in the middle ear. Anti-*bcl-*2 staining of the immature cochlear duct, sectioned tangentially, of 12-* (A) *and 15-week* (B) *fetuses show the presence of* bcl-*2 protein in sub-populations of neuroepithelial cells (anti-*bcl-*2,* ×*100).* C: *Note abundant* bcl-*2 protein in the cochlear hair cells (hc) of a 22-week fetus (anti-*bcl-*2,* ×*200).*

role in preserving this fetal tissue from death induced by the maternal immune system.

Many neurons degenerate and die in response to the death of cells with which they make synaptic connections (transsynaptic degeneration). Furthermore, many more neurons are produced during embryonic development than are present in the mature individual. Neurons that fail to establish functional synaptic relationships die.[21] Thus, evidence indicates that, to an important degree, neurons rely on trophic factors produced by the cells they innervate. In some instances this effect is mediated by nerve growth factor, but it is known that other substances are involved and likely that more remain to be discovered.[22] The frequent finding of abundant *bcl-2* protein in many neurons suggests that it may play a role in mediating the trophic effects that nerves have on one another. In this context, it is interesting that nerve growth factor protects dorsal root ganglion cells from death after sciatic nerve transection.[23] It is also interesting that the hair cells of the mature cochlea at 22 weeks showed abundant *bcl-2* expression, perhaps suggesting a role for cell death in the establishment of the extraordinarily complex and precise cellular interrelationships that are required for the formation of a functional organ for sound transduction.[24]

Cell death has long been recognized as an important, active process during human prenatal development, and its importance in a few processes has been well documented. Processes known to involve active cell death include the death of epithelial cells during fusion of the palatal shelves, the death of redundant interdigital tissue during limb development, and, as discussed above, the selective death of neurons that have failed to establish functional synaptic connections.[25] In this context, the presence of strong immunoreactivity for *bcl-2* in the fetal uterus and Fallopian tube stroma is interesting, particularly since it was not found in adult myometrium or endometrial stroma. Whereas in female fetuses, Müllerian-derived structures persist to develop into important components of the female genital tract, in males they regress and disappear under the influence of Müllerian-inhibiting substance.[26] Therefore, it is tempting to speculate that Müllerian-inhibiting substance might function, in part, by preventing *bcl-2* expression in the Müllerian ducts of male fetuses. Despite expression of *bcl-2* by smooth muscle at several other sites, myometrial staining is not merely a reflection of *bcl-2* expression by fetal smooth muscle in general, because vascular smooth muscle is devoid of staining, and staining in gastrointestinal muscularis propria is very weak. Also interesting is the presence of *bcl-2* protein in several components of the fetal eye, since cell death is known to play an important role in eye formation.[27]

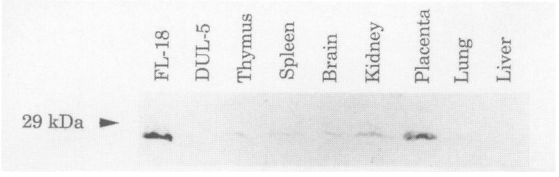


**Figure 7.** *Detection of* bcl-*2 protein by immunoblotting. As judged by the intensity of the single band at roughly 25 kd seen in lanes corresponding to most of the tissues studied,* bcl-*2 protein was most abundant in placenta, followed by kidney, brain, spleen, and thymus. A barely visible band corresponds to lung tissue. FL-18 is a t(14;18)-bearing cell line, and DUL-5 is a cell line without a 14;18 translocation.*

Inductive interactions between tissues are common during normal morphogenesis, and the initial morphological change in such events is often the formation of focal condensations of the induced cells. The formation of skin appendages is a good example. Animal experimentation has shown the initial step in this process to be induction of epidermal "placodes" by the underlying mesenchyme.[28] These basal condensations of keratinocytes then begin to grow downward into the dermis as epidermal buds and, in turn, induce condensation of dermal cells that grow into the deep aspect of the developing follicle as its mesenchymal core. We detected relatively abundant *bcl-2* protein in basal keratinocytes in epidermal buds and, at a later stage of follicle formation, in the cells of the mesenchymal core. In contrast, mitotic activity, as determined by ki-67 immunostaining on the frozen section, was abundant in the basal epithelial cells of the hair bulb and scant in the mesenchymal core. With the exception of epidermal buds, staining for *bcl-2* protein was weak or absent in the basal cell layer of the epidermis in 15- or 22-week fetuses.

The development of the tracheobronchial tree also requires inductive interactions between epithelial and mesenchymal elements.[29] In the developing lung, *bcl-2* expression was restricted to a small number of flattened, elongated mesenchymal cells immediately adjacent to the developing epithelial structures. The appearance and location of these altered mesenchymal cells suggested that they were destined to develop into the connective tissue components of the bronchial tree.

In the kidney, subcapsular condensations of mesenchymal cells adjacent to well-defined epithelial tubes showed strong *bcl-2* expression. These cells appear to correspond to those engaged in differentiating into the epithelial structures of proximal nephrons under the inductive influence of branches of the ureteric bud in a developmental interaction that has been well described.[30] Among epithelioid cells, the distribution of *bcl-2*-expressing cells was roughly reciprocal to those that were mitotically active.

Thus, the presence of *bcl-2* protein at several sites characterized by inductive interactions between epithelial and mesenchymal structures suggests that *bcl-2* might play a role in the "commitment" process undergone by groups of cells destined to participate in the formation of new structures. In this context, expression of *bcl-2*, possibly under the direct or indirect influence of soluble factors from nearby tissues, could contribute to the formation of the cellular condensations that are associated with the embryological formation of some structures by decreasing the focal rate of cell death relative to mitosis.

We used a single monoclonal antibody in this study. Therefore, despite the demonstrated specificity of this reagent, confirmation of our results with the use of other antibodies or techniques would be desirable. Despite this caveat, our study provides strong evidence that *bcl-2* is more widely expressed in the fetus than in the adult. More compellingly, the topographical distribution of *bcl-2* within several fetal tissues supports involvement of this protein in morphogenesis, in addition to tissue homeostasis, and is consistent with a role for enhanced cell survival (and possibly active cell death mechanisms) at many anatomical locations during fetal development.

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